THE INFLUENCE OF KAPPA-CASEIN AND BETA-LACTOGLOBULIN GENOTYPES ON MILK COAGULATION PROPERTIES IN LATVIA DAIRY BREED

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Abstract

Milk yield, composition and milk coagulation properties can be affected by kappa-casein (κ-CN) and beta-lactoglobulin (β-LG) genotypes and breed. Latvian Brown (LB) and Latvian Blue (LZ) are local dairy breeds in Latvia. These breeds are not so high-yielding than other commercial dairy breeds, besides, the number of those animals decrease each year. The aim of the research was to analyze the influence of κ-CN, β-LG and breed on milk composition and milk coagulation properties. Data were collected from 56 Latvian Brown and 26 Latvian Blue in 2016. Widespread κ-CN genotype was AA (0.593) in LB breed and AB (0.636) in LZ breed, while β-LG highest frequency was BB genotype. Significant effect was not observed on milk yield, however the highest daily milk yield was from AB κ-CN genotype (19.7 ± 1.52 kg) in LB breed. Significant differences were observed in fat content – the highest fat content accordingly κ-CN was from AA genotype in LB (46.3 ± 1.89 g kg^{-1}) and 45.5 ± 1.37 g kg^{-1} from BB β-LG genotype in LB breed ($p < 0.05$). A lower protein content was observed from AA κ-CN genotype in LZ breed (33.8 ± 1.30 g kg^{-1}), while highest from BB genotype in both breeds. Curd firmness was not significantly different by genotypes and breed. Significantly shorter milk renneting time of β-LG was obtained from LB breed (14.6 ± 2.76 min, $p < 0.05$), while κ-CN genotype was not significantly affected.

Key words: milk coagulation properties, kappa-casein, beta-lactoglobulin.

Introduction

Beta-lactoglobulin (β-LG) and kappa-casein (κ-CN) are two of the most important proteins in the milk of mammals (Flower, 1996). During the past 70 years, β-lactoglobulin has been studied by every available biochemical technique (Flower, North, & Sansom, 2000). The concentration of β-LG in bovine milk varied from 2 to 3 g kg^{-1} (Kontopidis, Holt, & Sawyer, 2004). Messenger RNA coding β-LG is synthesized in the mammary gland, and β-LG contains residues of 162 amino acids. Main alleles are A and B for coding this protein and only two amino acids are different. Aspartic acid is substituted with glycine at position 64 and valine with alanine at position 118 (Qin *et al*., 1999). Casein is milk protein which contains four casein fractions – α_{s1}-casein, α_{s2}-casein, β-casein and κ-casein. Two genetic κ-CN variants are more widespread – A and B. A variant has a threonine at position 136 and an aspartic acid at position 148 of the mature protein, while B variant has isoleicin and alanine residues at these positions, respectively (Alexander *et al*., 1988).

The coagulation ability of milk is essential for cheese making. Milk with favorable coagulation properties (short coagulation and curd firming time and a firm curd) is expected to give more cheese with desirable composition than milk with unfavorable properties. Scientists have found that 8% of milk samples do not coagulate during 30 min and haveanalyzed the factors which affect milk coagulation. Several researchers found that parity did not affect coagulation properties, but some authors found the opposite effect (Lindström, Antila, & Syväjarvi, 1984; Davoli, Dall'Olio, & Russo, 1990).

According to the results of the previous studies from Tsiaras and co-authors (2005), A of β-LG allele is more prevalent than B allele – it was found that genotype frequencies were 0.28, 0.47 and 0.25 for AA, AB and BB genotypes β-LG. A similar tendency was found by Kučerová *et al*., (2006). Scientists from Estonia found that A allele frequencies were 0.188, 0.138 and 0.319 for Estonian native, Estonian Red and Estonian Holstein, but B allele was found with frequency 0.813, 0.863 and 0.681, respectively (Värv *et al.,* 2009). According to the studies of Ren and his colleagues (2011), A allele and AA genotype of κ-CN are more widespread in Holstein and other high-yield dairy breeds, while the opposite situation was obtained for Jersey breed. The frequency of AA κ-CN genotype was 0.55 in Holstein, while this genotype was not observed in Jersey breed, but BB genotype of κ-CN with frequency 0.77 was observed in Jersey (Ren *et al*., 2011).

Schaar and co-authors (1985) found that the milk coagulation time was shorter for the β-LG BB genotype ($p < 0.001$), while the curd firmness was not affected by the β-LG genotypes. The β-LG AA genotype had a favorable effect on milk coagulation properties in some studies (Schaar, Hansson, & Pettersson, 1985; Aleandri *et al*., 1990 Ikonen *et al*., 1999; Wedholm *et al*., 2006). Pečiulaitienė and co-authors (2007) observed the highest milk yield and fat content from cows with AB genotype. Ng-Kwai-Hang and co-authors (1984, 1986) and Tsiaras with colleagues (2005) described the genotype κ-CN effect on milk yield, composition and milk coagulation properties.

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Animals with AB and BB κ-casein genotypes have the highest protein content and better milk coagulation properties.

Previous studies in Latvia have not described milk coagulation properties according to κ-casein and β-lactoglobolin genotypes.

The purpose of our research was to analyze κ-CN and β-LG genotypes and breed influence on milk composition and coagulation properties.

Materials and Methods

Data, blood samples and individual milk samples were collected from Latvian cow breeds (56 Latvian Brown (LB), 26 Latvian Blue (LZ)) from different regions of Latvia in 2016. Milk samples were collected during summer (July to August, 2016) period.

All cows were kept in small farms – on average 6 animals in each farm. Analyzed cows were grazed on pastures; grain meal and mineral additives were fed to them. LB cows were in 136 ± 8.1 lactation day and LZ – 144 ± 9.7 lactation day. Cows were milked twice. Average LB cows were 3.1 ± 0.17 parity and LZ – 3.5 ± 0.26 parity.

The blood samples were taken from the jugular vein (*Vena jugularis*) and collected in the K_3-EDTA coated sterile vacutainers, and stored at -20 °C until DNA extraction. The research was conducted at the Scientific Laboratory of Molecular Biology and Microbiology of Latvia University of Agriculture.

DNA was extracted by Genomic DNA Purification Kit# KO512 and DNEasy Blood@Tissue Kit (Fermentas, Lithuania) which was extracted by QIAcube (QIAcube, USA). β-LG alleles were identified using The PCR-RELP (Polymerase Chain Reaction and Restriction Fragment Length Polymorphism) according to Medrano and Aguilar - Cordova (1990) method.

Kappa-CN genotypes were identified using PCR-RFLP and electrophoresis on 3% agarose gel. The identification of κ-CN single nucleotide polymorphisms (SNPs) was done by methodology of Velmala *et al.* (1993). SNPs at positions 13104 and 13124 were examined to determine the nucleotide changes (A->C and A->G), which analyse κ-CN alleles A, B and also E. For digestion endonuclease *Hinf*I was used to detect the presence of alleles A and B, and endonuclease *BsuR*I (Fermentas, Lithuania) to detect the presence of the allele E.

Protein and fat content, milk density, curd yield and curd firmness, protein and fat ratio were detected at the Faculty of Food Technology of LUU. Protein content was detected according to ISO 8968-1:2014 using Kjeltec™ 2100 (Foss, Denmark). Fat content was detected according to ISO 488:2008 using centrifuge (Funke Gerber, Germany). Curd yield (%) was calculated by weight of curd obtained from milk.

Density of milk was measured according to Latvian Standard 186:1999 using lactodensitometer (Labtajm, Ukraine).

The rennet (CHY-MAX 1000 IMCU/mL, Chr. Hansen, Denmark) used in the analyses of curd firmness was diluted 1:100 (v/v) and added 0.2 mL to 10 mL of milk. The curd firmness (in Newton's) was determined after 30 min of milk renneting at 35 °C using Texture Analyser TA-HD plus (Stable Micro System, UK). Compression method for determination of curd firmness (technical data – disc A/BE – d45, test speed 1.0 mm s^{-1}, distance in the depth of curd sample 8 mm) was used.

Renneting time in minutes was analyzed using 1:100 (v/v) of rennet solution into water and measuring the time until flocculation of milk was started at 35 °C. For the interpretation of results, all samples were divided into four groups (fast, average, slow and non-coagulating milks) based on the time devoted to the clotting of samples. The assessment of clotting time was the following: fast means flocculation formation during 10 min, average – 15 min, slow – more than 15 min, and non-coagulating – samples that did not coagulate at all.

Analyses of milk composition and coagulation properties were not provided for AE κ-CN genotype taking into account the low number of cows with this genotype. In tables we have not included data about the milk composition and coagulation properties from cows with non-coagulated milk.

Statistical data processing was carried out using MS Excel and ANOVA of SPSS 15.0 for Windows. The differences between groups were significant if p<0.05 and were marked with letters in superscript ([a, b, c]) between genotypes and ([A,B]) between breeds.

Results and Discussion

We observed κ-CN allelic frequencies 0.778, 0.213 and 0.009 for A, B and E allele in LB breed (Table 1). Similar allelic frequencies of A and B alleles were found in LZ breed. E allele was not found in LZ breed. Widespread κ-CN genotype of LB breed was AA with frequency 0.593, while frequency of BB genotype was 0.037. AB κ-CN genotype frequency of LZ breed was 0.636, while AA genotype frequency was lower compared with LB breed – 0.318. A similar tendency of κ-CN allelic frequencies was observed by Pärna with colleagues (2007) in Estonian Holstein breed. Estonian native breed A, B and E allelic frequencies were 0.725, 0.238 and 0.038, respectively (Värv *et al.*, 2009).

B allele of β-LG genotype was widespread in both breeds – the frequencies were 0.917 and 0.886, respectively. Accordingly, BB genotype of β-LG was with frequencies 0.852 in LB breed and 0.773 in LZ

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Table 1

The frequencies of κ-CN and β-LG alleles and genotypes

Protein	Alleles and genotypes	Frequencies of alleles and genotypes	
		Latvian Brown (n = 56)	Latvian Blue (n = 26)
κ-CN	A	0.778	0.636
	B	0.213	0.364
	E	0.009	0.000
	AA	0.593	0.318
	AB	0.352	0.636
	BB	0.037	0.045
	AE	0.019	0.000
β-LG	A	0.083	0.114
	B	0.917	0.886
	AA	0.019	0.000
	AB	0.130	0.227
	BB	0.852	0.773

breed, while frequency of AA genotype was 0.019 and this genotype was not observed in LZ breed. Estonian native and Estonian Red frequencies of B β-LG allele were 0.813 and 0.863 (Värv *et al*., 2009).

Significant differences were not observed between κ-CN genotypes and breeds. The highest milk yield was observed from AB κ-CN genotype in LB breed (19.7 ± 1.52 kg), while a lower milk yield was BB genotype in the same breed (Table 2). Tsiaras *et al*., (2005) have found the same milk yield tendency – the highest milk yield was from AB genotype, an average from AA and a lower milk yield was obtained from BB genotype.

A significantly higher fat content was observed from AA genotype in LB breed (46.3 ± 1.89 g kg⁻¹) compared with LZ breed - 38.2 ± 3.63 g kg⁻¹ (p < 0.05). Different results were revealed in *Tsiaras et al.,* (2005) research – the highest fat content was observed from AB genotype. Lunden, Nilsson, & Janson (1997) had obtained no significant differences in fat content between the AA and AB genotypes.

Previous papers described a positive effect of B allele on protein content and protein yield (Ng-Kwai-Hang *et al*., 1984; Ng-Kwai-Hang, Monardes, & Hayes, 1990; Van Eenennaam & Medrano, 1991; Vallas *et al*., 2010). We did not find a significant difference, however a tendency appeared. The highest milk protein content was obtained from BB genotype in both breeds, while we found a lower protein content from AA genotype – 33.8 ± 1.30 g kg⁻¹ in LZ and 34.4 ± 0.68 in LB milk samples.

The average milk density (Table 2) was 1028 kg m⁻³ in all genotypes and breed, except milk from LZ cows with AB genotype. According to the studies of Wangdi and co-authors (2014), the milk

density can be affected by breed and vary from 1027 to 1030 kg m⁻³. Milk density influences milk chemical composition especially solids-not-fat (Jasińska *et al*., 2010).

Curd firmness (Table 2) was not significantly different between breeds, however, the highest curd firmness was obtained from AB genotype in LZ breed (3.35 ± 0.18 N), while a lower value was BB genotype in the same breed. Curd firmness is a very important factor, mainly because the amount of milk used for cheese manufacture is growing worldwide, and several works (De Marchi *et al*., 2008; Wedholm *et al*., 2006) have confirmed the importance of MCP as well as curd firmness on cheese processing, yield and quality.

In previous studies it was found that breed and genotypes (κ-CN and β-LG) can affect curd firmness significantly (Sturaro *et al*., 2012; Vallas *et al*., 2012).

A significantly higher curd yield was observed from BB κ-CN genotype in LB breed (28.4 ± 3.89%) and LZ breed (28.8 ± 6.16%, p < 0.05) compared with genotypes AA and AB. Allele B showed a positive effect on curd yield in both breeds. Ng-Kwai-Hang and co-authors (1984) have confirmed that milk obtained from cows with BB genotype has the highest curd yield compared to the milk collected from cows with AA and AB κ-casein genotype.

Milk renneting time (Table 2) was not significantly different between breeds and genotype, however longer time was observed from AB genotype in LZ breed (18.6 ± 4.44 min). Zannoni and Annibaldi (1981) recommended that optimal value of milk coagulation time is 13 min. We obtained a closer result from AB κ-CN in LB breed – 14.9 ± 1.23 min. Milk renneting time is influenced not only by genotype, but also by

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breed, season, milk composition and pH (Marchi *et al*., 2013).

Significant differences in protein to fat ratio were found in AB and BB genotypes, as well as in LZ breed (p < 0.05). Composition of the cheese and its yield can be significantly affected by changes in the concentration of protein and fat in milk. According to the study of Guinee and co-authors(2007), a ratio of protein to fat in milk between 0.77 and 0.83 (in case of Latvian Brown) results in a significantly less fat retention during the cheese production compared to cheese produced from milk with a ratio protein to fat between 0.95 and 1.21 (in case of Latvian Blue). The actual cheese yield reducing increased ratio protein to fat in milk and extensive impact was observed on reducing the moisture content of fat free matter, fat in dry matter, salt content (Guinee *et al*., 2007).

Analyzing influence of β-LG on milk yield in LB and LZ breeds, we did not observe significant differences. We observed different tendencies in breeds. The highest milk yield was observed from BB β-LG genotype in LB breed, while the highest milk yield was from AB genotype in LZ breed. Matějíček and co-authors (2008) found that the highest milk yield was associated with AA β-LG genotype.

Significant differences were obtained in fat content (p < 0.05; Table 3). A higher fat content was observed in AB β-LG genotype in LB compared with LZ breed. Significant differences were obtained in BB genotype – the highest fat content was found in BB genotype LB breed (45.5 ± 1.37 g kg^{-1}, p < 0.05). A positive effect on milk fat content by BB β-LG genotype was obtained in the study of Zaglool and co-authors (2016).

Protein content was not significantly different between research groups. The highest protein content

was obtained from BB genotype in LZ breed – 35.3 ± 1.19 g kg^{-1}.

Milk density was significantly different between LB and LZ breeds in AB β-LG genotype. The highest density was obtained in LZ breed (1030.4 ± 1.29 kg m^{-3}), while lower value was obtained in LB breed (1027.9 ± 0.14 kg m^{-3}, p < 0.05).

Milk coagulation properties are commonly defined by milk renneting time and curd firmness. A short milk coagulation time and a firmer curd are favorable for cheese production (Aleandri, Schneider, & Buttazzoni, 1989; Martin *et al*., 1997). Zannoni & Annibaldi (1981) study has revealed that the optimal milk renneting time is 13 minutes. Closer results we obtained from AB genotype in LB breed (14.6 ± 2.76 min).

Significant differences were not obtained in curd firmness and curd yield. The highest curd firmness was obtained from AB genotype in LB breed (3.79 ± 0.44 N), while lower curd firmness was from the same genotype in LZ breed (2.58 ± 0.52 N). The highest curd yield was observed from BB genotype – 24% in both breeds.

A significantly shorter milk renneting time was obtained from AB genotype in LB breed (14.6 ± 2.76 min), compared with LZ breed (21.7 ± 10.35 min, p < 0.05).

Protein to fat ratio was significantly different between genotypes in LZ breed. The highest protein to fat ratio was obtained from AB genotype (1.60 ± 0.39, p < 0.05) and this milk is not favorable for cheese making.

We observed average relationships between milk protein to fat ratio and curd yield. Phenotypical correlation coefficient was r_p = -0.56 in LB breed and r_p = -0.54 in LZ breed. According to Bojanić-Rašović

Table 2

Milk compositions and milk coagulation properties according to κ-CN

Trait	Latvian Brown			Latvian Blue		
	AA (n = 32)	AB (n = 19)	BB (n = 2)	AA (n = 7)	AB (n = 13)	BB (n = 2)
Daily milk yield, kg	19.6 ± 1.04	19.7 ± 1.52	14.6 ± 4.65	18.3 ± 1.14	16.7 ± 1.95	15.7 ± 7.67
Fat, g kg^{-1}	46.3 ± 1.89[a]	41.7 ± 2.04[a]	41.0 ± 3.37[a]	38.2 ± 3.63[b]	33.9 ± 3.24[b]	40.0 ± 5.39[b]
Protein, g kg^{-1}	34.4 ± 0.68	34.8 ± 0.81	36.7 ± 1.55	33.8 ± 1.30	35.5 ± 1.45	36.8 ± 1.48
Density, kg m^{-3}	1028.1± 0.25	1028.7 ± 0.25	1028.5 ± 0.20	1028.7 ± 0.50	1029.2 ± 0.67	1028.7 ± 0.42
Curd firmness, N	3.20 ± 0.24	3.22 ± 0.31	3.24 ± 0.65	3.17 ± 0.50	3.35 ± 0.18	2.73 ± 0.75
Curd yield,%	23.7 ± 0.94[a]	24.0 ± 1.66[a]	28.4 ± 3.89	21.4 ± 1.55[a]	23.5 ± 1.35[a]	28.8 ± 6.16
Milk renneting time, min	18.2 ± 1.99	14.9 ± 1.23	17.1 ± 1.39	17.7 ± 3.99	18.6 ± 4.44	17.4 ± 2.84
Protein to fat ratio	0.77 ± 0.03	0.83 ± 0.06[a]	0.77 ± 0.03[a]	0.95 ± 0.10	1.24 ± 0.17[b]	1.01 ± 0.08[b]

[a,b] – differences were significant between breeds in same κ-CN genotype (p < 0.05).

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Table 3

Milk compositions and milk coagulation properties according to β-LG

Trait	Latvian Brown		Latvian Blue	
	AB (n = 7)	BB (n = 46)	AB (n = 5)	BB (n = 17)
Milk yield, kg	18.5 ± 1.33	19.6 ± 0.93	18.0 ± 1.76	17.3 ± 1.57
Fat, g kg⁻¹	37.9 ± 4.46[a,A]	45.5 ± 1.37[a,B]	25.8 ± 5.22[b,A]	37.9 ± 2.39[b,B]
Protein, g kg⁻¹	34.2 ± 1.09	34.6 ± 0.55	33.7 ± 1.98	35.3 ± 1.19
Density, kg m⁻³	1027.9 ± 0.14[a]	1028.5 ± 0.20	1030.4 ± 1.29[b]	1028.7 ± 0.42
Curd firmness, N	3.79 ± 0.44	3.25 ± 0.19	2.58 ± 0.52	3.24 ± 0.27
Curd yield,%	22.9 ± 2.03	24.1 ± 0.89	19.3 ± 1.73	24.1 ± 1.16
Milk renneting time, min	14.6 ± 2.76[a]	17.1 ± 1.39	21.7 ± 10.35[b]	17.4 ± 2.84
Protein to fat ratio	0.96 ± 0.08	0.77 ± 0.03[a]	1.60 ± 0.39[A]	1.01 ± 0.08[b,B]

[a,b] – differences were significant between breeds in same β-LG genotype ($p < 0.05$).
[A,B] – differences were significant between β-LG genotypes in same breed ($p < 0.05$).
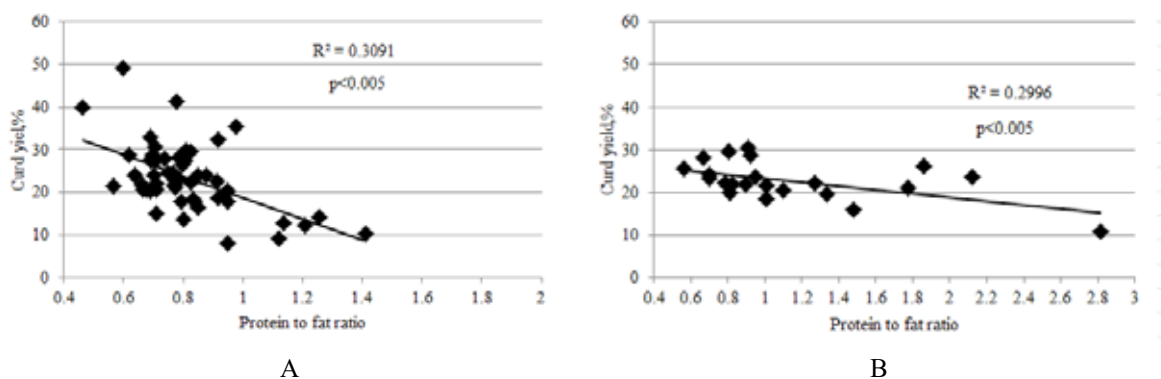

Figure 1. Relationships between milk protein to fat ratio and curd yield:
A – Latvian Brown and B – Latvian Blue breed.

and co-authors (2013), medium strong to no positive relationships were obtained between protein to fat ratio and curd yield ($r_p = -0.67$).

More extensive studies of milk coagulation properties could improve the knowledge about the Latvian local dairy breed and its milk perspectives for cheese making. Our results revealed BB and AB genotypes of κ-CN and β-LG positive effect on milk composition and coagulation properties. Therefore, for a better understanding and explanation of breed and genotype potential for cheese making further studies on milk protein profiles and genetic variants of different protein fractions during different seasons should be done.

Conclusions

Widespread κ-CN genotype was AA in LB breed and AB in LZ breed with frequencies 0.593 and 0.636, while the highest frequencies of β-LG was BB genotype.

Significant differences were observed in fat content – the highest fat content was in LB milk samples, accordingly, κ-CN was from AA genotype 46.3 ± 1.89 g kg⁻¹ – and 45.5 ± 1.37 g kg⁻¹ from BB β-LG genotype ($p < 0.05$).

Significantly shorter milk renneting time of β-LG was obtained from LB breed (14.6 ± 2.76 min, $p < 0.05$), while κ-CN genotype did not significantly affect this parameter.

A significantly higher curd yield was observed from BB κ-CN genotype – on average 28%, while β-LG genotype did not affect the curd yield significantly, however, the highest curd yield was obtained from BB genotypes in both breeds.

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